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**Amendment to the Specification:**

Please insert the pages of the Sequence Listing attached hereto as **Exhibit A** immediately preceding the Figures.

Please replace the following paragraph starting at page 24, line 2, with the following paragraph:

Nucleotide (a) and amino acid (b) sequences (SEQ ID NOs:17 and 18, respectively) for HIV-1<sub>JR-FL</sub> SOS gp140. The amino acid numbering system corresponds to that for wild-type JR-FL (Genbank Accession Number U63632). The cysteine mutations are indicated in underlined bold type face.

Please replace the following paragraph starting at page 24, line 10, with the following paragraph:

Nucleotide (a) and amino acid (b) sequences (SEQ ID NOs:19 and 20, respectively) for HIV-1<sub>JR-FL</sub> ΔV1V2\* SOS gp140. The amino acid numbering system corresponds to that for wild-type JR-FL (Genbank Accession Number U63632). The cysteine mutations are indicated in underlined bold type face.

Please replace the following paragraph starting at page 24, line 17, with the following paragraph:

Nucleotide (a) and amino acid (b) sequences (SEQ ID NOs:21 and 22, respectively) for HIV-1<sub>JR-FL</sub> ΔV3 SOS gp140. The amino acid numbering system corresponds to that for wild-type JR-FL (Genbank Accession Number U63632). The cysteine mutations are indicated in underlined bold type face.

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Please replace the following paragraph starting at page 45, line 1, with the following paragraph:

HIV-1<sub>JR-FL</sub>, the C1 region consists of the amino acids VEKLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNWATHACVPTDPNPQEVVLENV TEHFNMWKNNMVEQM**QEDIISLWDQSLKPCVKLTPLCVTLN** (SEQ ID NO:1). Amino acid residues 30-130 of the sequence set forth in Figure 3a have this sequence. In other HIV isolates, the C1 region will comprise a homologous amino-terminal sequence of amino acids of similar length. W44C and P600C mutations are as defined above for A492 and T596 mutations. Because of the sequence variability of HIV, W44 and P600 will not be at positions 44 and 600 in all HIV isolates. In other HIV isolates, homologous, non-cysteine amino acids may also be present in the place of the tryptophan and proline. This invention encompasses cysteine mutations in such amino acids, which can be readily identified in other HIV isolates by those skilled in the art.

Please replace the following paragraph starting at page 45, line 17, with the following paragraph:

As used herein, "C5 region" means the fifth conserved sequence of amino acids in the gp120 glycoprotein. The C5 region includes the carboxy-terminal amino acids. In HIV-1<sub>JR-FL</sub> gp120, the unmodified C5 region consists of the amino acids GGGDMRDNRSELKYKVVKIEPLGVAPTKAKRRVVQRE (SEQ ID NO:2). Amino acid residues 462-500 of the sequence set forth in Figure 3a have this sequence. In other HIV isolates, the C5 region will comprise a homologous carboxy-terminal sequence of amino acids of similar length.

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Please replace the following paragraph starting at page 66, line 25, with the following paragraph:

*Wild-type gp140s (gp140WT).* The gp140 coding sequences were amplified using the polymerase chain reaction (PCR) from full-length molecular clones of the HIV-1 isolates JR-FL, DH123, Gun-1, 89.6, NL4-3 and HxB2. The 5' primer used was designated Kpn1env (5'-GTCTATTATGGGGTACCTGTGTGGAA AGAAGC-3') (SEQ ID NO:3) while the 3' primer was BstB1env (5'-CGCAGACGCAGATTGAATT AATACCACAGCCAGTT-3') (SEQ ID NO:4). PCR was performed under stringent conditions to limit the extent of Taq polymerase-introduced error. The PCR products were digested with the restriction enzymes Kpn1 and Xho1 and purified by agarose gel electrophoresis. Plasmid PPI4-tPA-gp120JR-FL was also

Please replace the following paragraph starting at page 67, line 17, with the following paragraph:

*gp140UNC.* A gp120-gp41 cleavage site mutant of JR-FL gp140 was generated by substitutions within the REKR motif at the gp120 C-terminus, as described previously (Earl, 1990). The deletions were made by site-directed mutagenesis using the mutagenic primers 5'140M (5'-CTACGACTTCGTCTCCGCCTCGACTACGG GGAATAGGAGCTGTGTTCCCTGGGTCTTG-3') (SEQ ID NO:5) and 3'gp140M (sequence conjunction with Kpn1env and BstB1env 5' - CGAAGGCCGGAGACGAAGTCGTAGCCGCAGTGCCTTGGTGGGTGCTACTCCTAATGGTTC -3') (SEQ ID NO:6). In conjunction with Kpn1env and BstB1, the PCR product was digested with Kpn1 and BstB1 and subcloned into pPPI4 as described above.

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Please replace the following paragraph starting at page 68, line 4, with the following paragraph:

PCR amplification using DGKPN5'PPI4 and 5JV1V2-B (5'-GTCTATTATGGGGTACCTGTGTGGAAAGAAGC-3') (SEQ ID NO:7) on a  $\Delta$ V1 template and subsequent digestion by Kpn1 and BamH1 generated a 292bp fragment lacking the sequences encoding the V1 loop. This fragment was cloned into a plasmid lacking the sequences for the V2 loop using the Kpn1 and BamH1 restriction sites. The resulting plasmid was designated  $\Delta$ V1V2' and contained a Gly-Ala-Gly sequences in place of both D132-K152 and F156-I191. Envs lacking the V1, V2 and V3 loops were generated in a similar way using a fragment generated by PCR on a  $\Delta$ V3 template with primers 3JV2-B (5'-GTCTGAGTCGGATCCTGTGA CACCTCAGTCATTACACAG-3') (SEQ ID NO: 8) and H6NEW (5'CTCGAGTCTCGAATTAGTGATGGTGATGGTGATGATAACCACAGCCATTTGTTATGTC-3') (SEQ ID NO:9). The fragment was cloned into  $\Delta$ V1V2', using BamH1 and BstB1. The resulting env construct was named  $\Delta$ V1V2'V3. The glycoproteins encoded by the  $\Delta$ V1V2' and  $\Delta$ V1V2'V3 plasmids encode a short sequence of amino acids spanning C125 to C130. These sequences were removed using mutagenic primers that replace T127-I191 with a Gly-Ala-Gly sequence. We performed PCR amplification with primers 3'DV1V2STU1 (5'-GGCTCAAAGGATATCTTGGACAGGCCTGTGTAATGACTGAGGTGTCACATCCTGCACCACAGAGTGGGTTAATTACACATGGC-3') (SEQ ID NO:10) and DGKPN5'PPI4, digested the resulting fragment by Stu1 and Kpn1 and cloned it in a PPI4 gp140 vector. The resulting gp140 was named  $\Delta$ V1V2\*. In an analogous manner  $\Delta$ V1V2\*V3 was constructed. The amino acid substitutions are shown schematically in Figure 10.

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Please replace the following paragraph starting at page 70, line 1, with the following paragraph:

*ELISA analyses.* The concentration of gp120 and gp140 proteins in 293T cell supernatants was measured by ELISA (Binley, 1997b). Briefly, Immulon II ELISA plates (Dynatech Laboratories, Inc.) were coated for 16-20 hours at 4°C with a polyclonal sheep antibody that recognizes the carboxy-terminal sequence of gp120 (APTKAKRRVVQREKR) (SEQ ID NO:11). The plate was washed with tris buffered saline (TBS) and then blocked with 2% nonfat milk in TBS. Cell supernatants (100µL) were added in a range of dilutions in tris buffered saline containing 10% fetal bovine serum. The plate was incubated for 1 hour at ambient temperature and washed with TBS. Anti-gp120 or anti-gp41 antibody was then added for an additional hour. The plate was washed with TBS, and the amount of bound antibody is detected using alkaline phosphatase conjugated goat anti-human IgG or goat anti-mouse IgG. Alternatively, biotinylated reporter Antibodies are used according to the same procedure and detected using a streptavidin-AP conjugate. In either case, AP activity is measured using the AMPAK kit (DAKO) according to the manufacturer's instructions. To examine the reactivity of denatured HIV envelope proteins, the cell supernatants were boiled for 5 minutes in the presence of 1% of the detergents sodium dodecyl sulfate and NP-40 prior to loading onto ELISA plates in a range of dilutions. Purified recombinant JR-FL gp120 was used as a reference standard.

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Please replace the following paragraph starting at page 89, line 6, with the following paragraph:

*Plasmids.* The pPPI4 eukaryotic expression vectors encoding SOS and uncleaved forms of HIV-1<sub>JR-FL</sub> gp140 have been described previously (Binley, 2000a; and Trkola, 1996). The SOS gp140 protein contains cysteine substitutions at residues A501 in the C5 region of gp120 and T605 in gp41 (Binley, 2000a; and Sanders, 2000). In gp140UNC, the sequence KRRVVQREKRAV (SEQ ID NO:12) at the junction between gp120 and gp41ECTO has been replaced with a hexameric LR motif to prevent scission of gp140 into gp120 and gp41ECTO (Binley, 2000a). Plasmids encoding variable-loop-deleted forms of HIV-1<sub>JR-FL</sub> SOS gp140 have been described (Sanders, 2000). In these constructs, the tripeptide GAG is used to replace V1 loop sequences (D133-K155) and V2 loop sequences (F159-I194), alone or in combination. The SOS gp140UNC protein contains the same cysteine substitutions that are present in SOS gp140, but the residues REKR (SEQ ID NO:13) at the gp120-gp41ECTO cleavage site have been replaced by the sequence IEGR (SEQ ID NO:14), to prevent gp140 cleavage. The furin gene (Thomas, 1988) was expressed from plasmid pcDNA3.1furin (Binley, 2000a).

Please replace the following paragraph starting at page 89, line 26, with the following paragraph:

*MAbs and CD4-based proteins.* The following anti-gp120 MAbs were used: IgG1b12 [against the CD4 binding site (Burton, 1994)], 2G12 [against a unique C3-V4 glycan-dependent epitope (Trkola, 1996)], 17b [against a CD4-inducible epitope (Thali, 1993)], 19b [against the V3 loop (Moore,

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1995)], and 23A [against the C5 region (Moore, 1996)]. The anti-gp41 MAbs were 2F5 [against a cluster 1 epitope centered on the sequence ELDKWA (SEQ ID NO:15) (Muster, 1993; and Parker, 2001)] and 2.2B [against epitope cluster II].

MAbs

IgG1b12,

Please replace the following paragraph starting at page 124, line 22, with the following paragraph:

*ELISPOT assay.* HIV-gp120 specific T cells are quantified using an IFN $\gamma$ -ELISPOT assay, essentially as described (Miyahira, 1995). Briefly, mixed cellulose ester membrane 96-well plates (Millipore) are coated with an anti-mouse anti-IFN $\gamma$  antibody (5 $\mu$ g/ml; MABTech) for 2 hours at 37°C and washed thrice in PBS. The wells are blocked in complete RPMI medium (RPMI 1640,  $\alpha$ -MEM, FBS (10%, Gibco) HEPES (10mM Gibco), L-Gln (2mM), 2-mercaptoethanol (50 $\mu$ M) for a further 2 hours at 37°C. After washing the wells thrice with PBS, single cell suspensions of splenocytes are added at 1-5  $\times$  10<sup>5</sup> cells per well in the presence of gp120 protein (5 $\mu$ M) or H-2<sup>d</sup> restricted gp120 peptide (RGPGRAFVTI (2 $\mu$ M)) (SEQ ID NO:16) for 16-20 hours.